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ORIGINAL PAPER

Development *trans-N*-benzyl hydroxyl cinnamamide based compounds from cinnamic acids and characteristics anticancer potency

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Abstract
The derivatization of three hydroxycinnamamides becomes *trans-N*-benzylhydroxycinnamamides, and their potential assay as anticancer agents has been carried out. *N*-benzyl-*p*-coumaramide (**5a**), *N*-benzylcaffeamide (**5b**), and *N*-benzylferulamide (**5c**) were obtained from *p*-coumaric, caffeic, and ferulic acid, respectively, with benzylamine via four reaction steps, i.e., acetylation, chlorination, amidation, and deacetylation. All products characterize using FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy, and their cytotoxicity were tested against P388 leukemia murine cells by MTT method. Although compound **5b** and **5c** have no and low anticancer activity with IC_{50} sequentially of 674.38 and 179.56 µg/mL, compound **5a** showed potentially use as an anticancer agent with IC_{50} of 16.15 µg/mL Molecular modelling studies were performed to understand the interactions with the activity against murine leukemia P388 cells.

Keywords Cytotoxic · Hydroxycinnamamides · Hydroxycinnamic · Leukemia · *N*-Benzyl · P388 cells

Introduction

The hydroxycinnamic acid compounds (e.g., *p*-coumaric, ferulic, and caffeic acids) as a new drugs or agent is interesting research topic of scientist in the world due to the antioxidant [1–3, 12, 19, 30, 36, 38, 41], antiviral [4], anticancer [1, 23, 31], antimicrobial [2, 12, 14, 21, 30], and antifungal activities [22, 44, 46] of their ester and amide derivatives. The activities are thought to arise from *p*-hydroxycinnamoyl and amine moiety in amides or alcohol moiety in ester [3], coupled with the presence of phenolic nuclei conjugated with the olefin group of the carbonyl. Such a molecular framework will facilitate the resonance of phenoxy radicals, thus it becomes relatively stable [12]. In addition, the α,β-unsaturated carbonyl group which can be considered as Michael's acceptor, with an active moiety commonly used in designing anticancer drugs [18, 26].

Esters and amides of hydroxycinnamic acids are more active than their free acid [12], so most of the researchers did the conversion of cinnamic hydroxyl compounds into their ester and amides before testing their bioactivity [3, 4, 6, 14, 33, 42]. The esterification and amidation of hydroxycinnamic acids are practical techniques to improve their solubility and emulsification properties as well as evaluated as potential antioxidant and antidiabetic activities [40, 45].

Although many hydroxycinnamic acids derived ester analogs display the potential activities, the ester groups in hydroxycinnamic acid are metabolically more labile than amides and have limited use [29]. In this study, we synthesized three hydroxycinnamide compounds from *p*-coumaric, caffeic and ferulic acid, and benzylamine to produce *N*-benzyl-*p*-coumaramide (**5a**), *N*-benzylcaffeamide (**5b**), and *N*-benzylferulamide (**5c**), respectively, as well as evaluated their cytotoxic activity against P388 murine leukemia cells. Based on the IC_{50} value, compound **5a** was the most active between the synthesized compounds. Moreover, it was more active than *N*-(*o*-tolyl)-*p*-coumaramide, which

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was previously synthesized [26], and potentially used as an anticancer drug.

Materials and method

Materials

p-Coumaric acid, caffeic acid, ferulic acid, benzylamine, acetic anhydride (Ac_2O), thionyl chloride, pyridine, triethylamine (TEA), 4-(dimethylamino)pyridine (DMAP), pyrrolidine, dichloromethane (DCM), ethyl acetate (EtOAc), benzene, methanol, *n*-hexane, NH_4Cl, HCl, and anhydrous Na_2SO_4 were pro synthesis and pro analysis grades and were used without purification. The FTIR spectra were recorded on a Spectrophotometer Shimadzu Prestige 21, the ^{1}H-NMR and ^{13}C-NMR spectra were collected using Spectrometer Agilent operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C nuclei, and the melting points were taken using electrothermal apparatus.

Synthesis method

The synthesis of **5a**, **5b,** and **5c** from *p*-coumaric, caffeic, and ferulic acid, and benzylamine here were conducted according to the procedure in reference [9]. This procedure consists of four stages of reactions, i.e., (1) acetylation reaction to protect the phenolic groups of each hydroxycinnamic acids gave acetic acetoxycinnamic anhydrides (**2a–c**), (2) chlorination reaction to activate the carboxyl group of the acetylation products gave acetoxycinnamoylchlorides, used for amidation without any purification (**3a–c**), (3) amidation reaction of the chlorination products using benzylamine to obtain *N*-benzylacetoxycinnamamides (**4a–c**), and (4) deacetylation reaction using pyrrolidine to deprotect the hydroxyl group of the amidation products gave *N*-benzylhydroxycinnamamides (**5a–c**) as the target compounds.

Acetic *p*-acetoxycinnamic anhydride 2a

A yellowish crystalline solid with m.p. of 205–207 °C (71.0% of yield), FTIR (KBr): υ (cm^{-1}) 3047.53 (C–H unsaturation), 2981.95 and 2827.64 (C–H saturation), 1745.58 (C=O acetyl), 1687.71 (C=O conj.), 1625.99 (C=C olefin), 1598.99 and 1506.41 (C=C Ar), 1427.32 and 1371.39 (CH_3), 991.41 (*trans*-olefin), and 839.03 (*p*-substance Ar).

Acetic 3,4-diacetoxycinnamic anhydride 2b

A white crystalline solid with m.p. of 184–185 °C (83.2% of yield), FTIR (KBr): υ (cm^{-1}) 3024.38 (C–H unsaturation.), 2985.81 (C–H saturation), 1764.87 (C=O acetyl), 1687.71 (C=O conj.), 1631.78 (C=C olefin), 1610.56 and 1504.48 (C=C Ar), 1431.18 and 1373.32 (CH_3), 983.70 (*trans*-olefin), and 908.47 and 827.46 (tri-substance. Ar).

Acetic 3-metoxy-4-acetoxycinnamic anhydride 2c

A white crystalline solid with m.p. of 182–184 °C (82.3% of yield), FTIR (KBr): υ (cm^{-1}) 3010.88 (C–H unsaturation), 2945.30 (C–H saturation), 1761.01 (C=O acetyl), 1687.71 (C=O conj.), 1631.78 (C=C olefin), 1600.92 and 1506.41 (C=C Ar), 1419.61 and 1371.39 (CH_3), 985.62 (*trans*-olefin), and 933.55 and 856.39 (tri-substance Ar).

N-benzyl-*p*-acetoxycinnamamide 4a

A yellowish solid, FTIR (KBr): υ (cm^{-1}) 3290.56 (*sec.* N–H), 3062.96 and 3002.32 (C–H unsaturation), 2852.72 and 2922.16 (C–H saturation), 1761.01 (C=O ester), 1654.92 (C=O amide), 1622.13 (C=C olefin), 1537.27 (C–N amide), 1504.48 (C=C Ar), 1367.53 (N–C), 972.12 (*trans*-olefin), 844.82 (*p*-substance Ar), 700.16 and 746.45 (mono-substance Ar).

N-benzyl-3,4-diacetoxycinnamamide 4b

A white crystalline solid with m.p. of 170–174 °C, FTIR (KBr): υ (cm^{-1}) 3265.49 (*sec.* N–H), 3068.75 (C–H unsaturation), 2933.73 (C–H saturation), 1774.51 (C=O ester), 1656.85 (C=O amide), 1616.35 (C=C olefin), 1548.84 (C–N amide), 1504.48 (C=C Ar), 968.27 (*trans*-olefin), 966.34 and 829.39 (tri-subst. Ar), 700.16 and 742.59 (mono-subst. Ar).

N-benzyl-3-methoxy-4-acetoxycinnamamide 4c

A white crystalline solid with m.p. of 118–122 °C, FTIR (KBr): υ (cm^{-1}) 3184.48 (*sec.* N–H), 3080.75 (C–H unsaturation), 2941.44 (C–H saturation), 1762.94 (C=O ester), 1656.85 (C=O amide), 1656.85 (C=C olefin), 1552.70 (C–N amide), 1512.19 (C=C Ar), 987.55 (*trans*-olefin), 902.69 and 854.47 (tri-substance Ar), 700.16 and 756.10 (mono-substance Ar).

N-benzyl-*p*-coumaramide 5a

A white crystalline with m.p. of 108–110 °C (76.9% of yields), FTIR (KBr): υ (cm^{-1}) 3423.65 (O–H), 3300.20 (N–H), 3022.45 (C–H unsaturation), 1653.00 (C=O amide), 1610.56 (C=C olefin), 1514.12 (C=C Ar), 1546.91 (N–C), 977.91(*trans*-olefin), 829.39 (*p*-subst. Ar), 698.23 and 732.96 (mono substance). ^{1}H-NMR (500 MHz, acetone-d_6) δ, ppm: 4.51 (2H, *d, J*=6 Hz, H-1′), 6.58 (1H, *d, J*=15.7 Hz, H-2), 6.86 (2H, *d, J*=8.55 Hz, H-6), 7.23 (1H, *t, J*=14 Hz,

H-5′), 7.32 (3H, *m*, H-3′ dan H-4′), 7.44 (2H, *d, J*=8.6 Hz, H-5), 7.53 (1H, *d, J*=15.65 Hz, H-3), 7.71 (1H, *b.s,* NH), 8.88 (OH). ^{13}C-NMR (100 MHz, acetone-d_6) δ, ppm: 43.64 (C-1′), 116.56 (C-6), 119.35 (C-2), 127.65 (C-5′), 127.71 (C-3′), 128.40 (C-4), 129.18 (C-4), 130.17 (C-4′), 140.57 (C-3), 140.63 (C-2′), 159.79 (C-7), 166.58 (C-1).

N-benzylcaffeamide 5b

A white crystalline with m.p. of 140–143 °C (78.8% of yields), FTIR (KBr): υ (cm^{-1}) 3479.58 and 3234.62 (O–H), 3305.99 (N–H), 3032.10 (C–H unsaturation), 1656.85 (C=O amide), 1593.20 (C=C olefin), 1500.00 (C=C Ar), 1543.05 (N–C), 970.19 (*trans*-olefin), 850.61 and 813.96 (tri-substance Ar), 742.59 and 696.30 (mono substance). ^{1}H-NMR (500 MHz, methanol-d_4) δ, ppm: 4.47 (2H, *s*, H-10), 4.85 (2H, *s*, O–H phenolic), 6.41 (1H, *d, J* = 15.5 Hz, H-2), 6.76 (1H, *d*, H-8), 6.91 (1H, *d*, H-9), 7.01 (1H, *s*, H-5), 7.1–7.2 (1H, *b.s*, N–H amide), 7.24–7.31 (5H, *m*, H-12, H-13, H-14, H-15, and H-16), 7.43 (1H, *d*, *J* = 15,4 Hz, H-3). ^{13}C-NMR (100 MHz, methanol-d_4) δ, ppm: 44.26 (C-10), 115.10 (C-5), 116.43 (C-8), 118.23 (C-2), 122.13 (C-9), 128.20 (C-4), 128.28 (C-12 and C-16), 128.59 (C-13 and C-15), 129.54 (C-14), 140.01 (C-11), 142.56 (C-3), 146.68 (C-6), 148.77 (C-7), and 169.16 (C-1).

N-benzylferulamide 5c

A white crystalline with m.p. of 120–122 °C (83.31% of yields), FTIR (KBr): υ (cm^{-1}) 3493.09 (O–H), 3250.05 (N–H), 3084.18 (C–H unsaturation), 1653.00 (C=O amide), 1606.70 (C=C olefin), 1508.33 (C=C Ar), 1537.27 (N–C), 974 (*trans*-olefin), 923.83 and 815.89 (tri-subst. Ar), 758.02 and 700.16 (mono substance). ^{1}H-NMR (500 MHz, chloroform-*d*) δ, ppm: 3.87 (3H, *s*, H-17), 4.58 (2H, *d, J* = 5.75 Hz, H-10), 6.01 (1H, *s*, OH phenolic), 6.29 (1H, *d, J* = 15.6 Hz, H-2), 6.89 (1H, *d, J* = 8.2 Hz, H-8), 6.96 (1H, *s*, H-5), 7.03 (1H, *dd, J* = 8,2 Hz, H-9), 7.26–7.27 (1H, *s*, N–H amide), 7.28 (2H, *m*, H-12 and H-16), 7.30 (1H, *m*, H-14), 7.31 (2H, *m*, H-13 and H-15), 7.56 (1H, *d, J* = 15.6 Hz, H-3). ^{13}C-NMR (100 MHz, chloroform-*d*) δ, ppm: 43.97 (C-10), 56.02 (C-17), 109.82 (C-5), 114.90 (C-8), 118.09 (C-2), 122.23 (C-9), 127.41 (C-4), 127.67 (C-12 and C-16), 128.02 (C-13 and C-15), 128.85 (C-14), 138.41 (C-11), 141.55 (C-3), 146.87 (C-6), 147.60 (C-7), 166.26 (C-1).

Bioactivity assay against P388 leukemia murine cells

The activity of compound **5a–c** against P388 leukemia murine cells were analyzed using an MTT method according to the procedure in references [24, 35].

Docking studies

Preparation of protein receptor and ligand standard

Protein receptor human DNA topoisomerase (Top1) was downloaded from Protein Data Bank webpage https://www.rcsb.org/structure/1T8I. In order to obtain the active site of this protein, the coordinate of Campthothecin (Cpt) was retrieved from the protein complexes. All of residues were removed then prepared to dock in Chimera software [32]. Likewise, Campthothecin (Cpt) as a standard ligand was extracted from the protein complexes and saved as.pdb format file.

Preparation of new ligands

There were two ligands **5a** and **5b** that docked into the active site of Top1. This two ligands then built using Avogadro software [15] and optimized in Chimera software using AM1-BCC semi empirical method then saved as.pdb format file.

Molecular docking analysis

Docking analysis was exhibit using AutoDock 4.2 software with the help of AutoDock Tools program [28]. Each ligand was docked into the active site of Top1 protein receptor. Grid box size was set 40 × 40 × 40 Å with spacing 0.375 Å and saved as parameter file in.gpf format file. Docking procedure was set to produce 20 conformations and run for maximum energy evaluation of 25,000,000. The Lamarckian Genetic Algorithm was used to obtain data in the form of binding energy (kcal/mol) and predicted inhibition constant. Validation of docking was determined from the value of Root Mean Square Deviation (RMSD) of redocking standard ligand (Cpt) into the active site of protein receptor. Successful redocking process was known from low RMSD value about less than 2 Å [17]. Visualization of docking result was done by using Discovery Studio Visualizer software for windows [5]. LigPlot + program was used to analyze the hydrophobic interactions of ligands against Top1 protein receptor [25].

Results and discussion

Chemistry

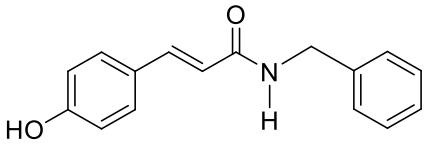
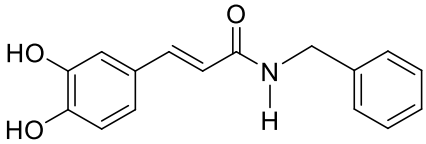
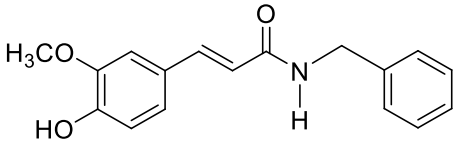
Boric acid has been successfully catalyzed the conversion of 4-phenylbutyric acid to *N*-benzyl-4-phenylbutyramide using benzylamine [39]. However, this catalyst failed to catalyze the conversion of hydroxycinnamic acid to its amide [8]. This failure is probably caused by the presence of a conjugate system in the hydroxycinnamic

acid structure and hydroxyl group on the phenolic moiety. Therefore, the conversion reaction must conduct by other methods. There are two general methods to convert hydroxycinnamic acid to an ester and amide, (1) directly conversion using a catalyst [27, 34, 39, 43], (2) indirect method involves four synthetic steps: (a) protection of the phenolic hydroxyl group, (b) activation of the carboxylic acid group, (c) condensation with alcohols or amines, and (d) deprotection of the phenolic hydroxyl group [16, 20, 27].

In this research, the synthesis of the target compounds was conducted by the indirect method (Fig. 1). The protection of the phenolic group in hydroxycinnamic acids (**1a–c**) were performed using acetic anhydride and produced acetoxycinnamic acids (**2a–c**). In addition, the conversion reaction of compound **2a–c** into *N*-benzylacetoxycinnamamides (**4a–c**) were performed through chlorination, and amidation reactions in situ generating a yellowish paste. The resulted compounds were then purified by column chromatography and continued by deprotection to gave *N*-benzylhydroxycinnamamides (**5a–c**) as white crystalline. The structure of synthesized compounds with the yields from the last reaction step are given in Table 1.

The success of pyrrolidine in 95% ethanol in removing the acetyl protecting groups without affecting the hydroxycinnamate bond is quite convenient [16]. Therefore, the method still applied in this research. All the main products in this synthesis reaction stages were analyzed by FTIR spectrometer, except for the chlorination products.

Table 1 The structure and yield of each synthesized compounds

Symbol	Compound structure	Yield (%)
5a	 N-benzyl-p-coumaramide	76.9
5b	 N-benzylcaffeamide	78.8
5c	 N-benzylferulamide	83.31

Furthermore, deacetylation products were analyzed using ^{1}H-NMR and ^{13}C-NMR spectrometers.

The FTIR spectra of acetylation products no longer showed any O–H absorption band, either O–H of phenolic or O–H of carboxylate. This data means, acetylation did not only occur in phenolic OH groups but also

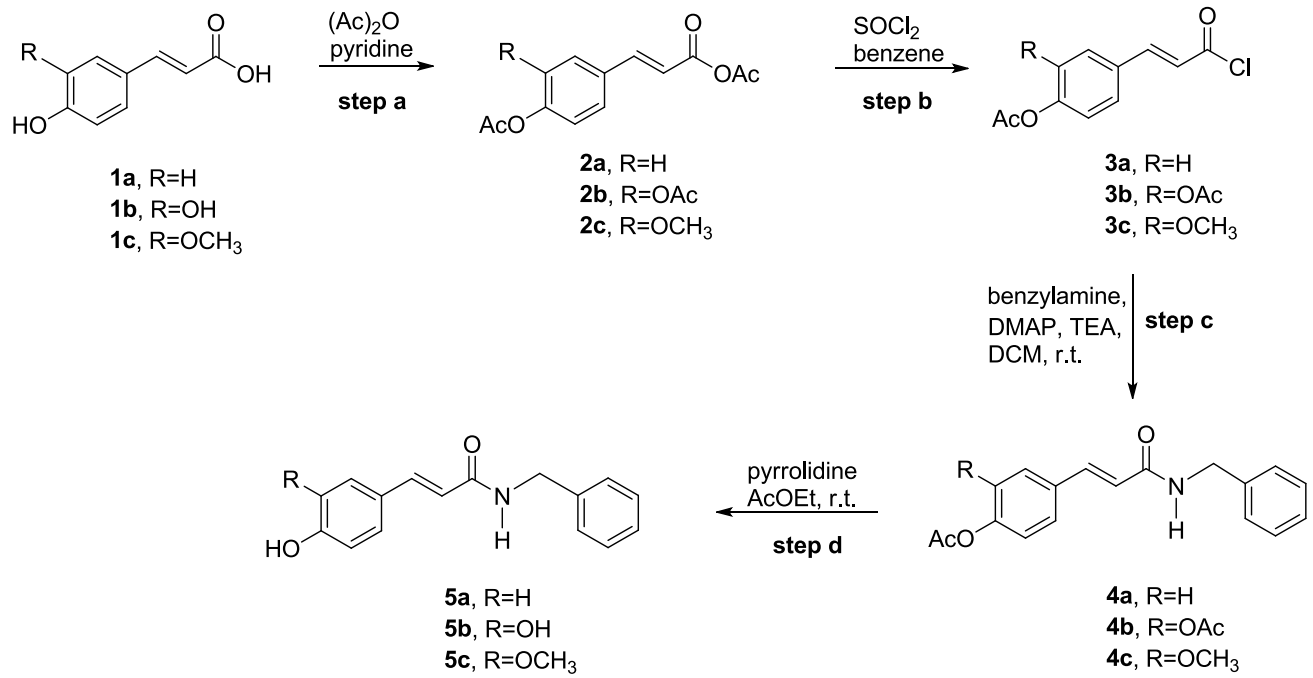


Fig. 1 The pathway of synthesis reaction of *trans*-*N*-benzylhydroxycinnamamides

occurred in carboxylic groups. Besides, the spectra also show the absorption of carbonyl esters that appear around 1745–1761 cm^{-1}, which indicate the presence of the acetyl group in the compounds. All of this data indicate the success of the acetylation reaction.

The success of the in situ amidation reaction was indicated by the presence of a sharp band with strong intensity in each spectrum around 1650 cm^{-1} as an amide I band and a sharp band with medium intensity from N–H bond at around 3184–3290 cm^{-1} as an amide band II in the FTIR spectrum of the reaction products. When the amidation products were deacetylated, the crystalline solid products were obtained.

NMR spectra of all the compounds shows different substituent –C (C-1 to C-l6), and –H (H-2 to H-14) have been analyzed from their NMR spectra. We have performed the analysis with a 1D experiment like 1 H, 13C, and compare chemical shifts with different substituents at C (3), 1 H, 13C NMR of all compounds. The 1 H chemical shifts of all three series (51, **5b**, and **5c**) of compounds obtained from the 1 H, 13C NMR spectra have been compared. 1 H chemical shift of –NH– was observed for H-2 to H-14, the X-ray crystal structures of these compounds reveal intra and intermolecular N–H···O interactions. Besides, the C (3) substitution may affect the 1 H chemical shift of –NH-. The downfield 2 H chemical shift was noticed for the H-2 and C-2. The aromatic proton H(5) takes part intermolecular C–H···O interaction in most of the crystal structures of derivatives. The 1 H chemical shift implies that the intermolecular C–H···O interaction. The differences for the 13C chemical shift of C(2), C(10), C(12), and C(13) of the series are transparent. The chemical shift for C-2 to C-14, showed the carbon chemical shift. For proton (H-2 to H-14) which ascertains the intramolecular hydrogen bonding between C–O–N–H is stronger in hydrogen. The differences observed in the carbon's chemical shift may be due to the electronegativity differences. The 13C chemical shift of *n*-alkylamino carbons of analogs compounds was found in a similar range except for the terminal methyl carbons. The variation in chemical shift can be correlated to hydrogen bond strength (intra and intermolecular) and the presence of electronegative substituent.

Cytotoxicity study

Many of ester and amide derivatives of hydroxycinnamic acid have been synthesized and tested for their cytotoxic against P388 [7, 9–11]. According to the IC_{50} values of *trans*-3-(4-hydroxy-3-methoxyphenyl)acrylate (10.79 µg/mL) and *trans*-3-(4-hydroxy-3-methoxyphenyl)-*N*-phenethylacrylamide (29.14 µg/mL), the ester compound was more active than amide compound [7]. However, the amide compound should more effective than the ester analogue due to the stability of the amide compounds. Furthermore, the IC_{50} of both *N*-(*o*-tolyl)caffeamide (0.91 µg/mL) and *N*-(*o*-tolyl)-*p*-coumaramide (16.97 µg/mL) (Fig. 2) indicated that *p*-hydroxycinnamoyl group has a significant effect on the activity against P388 murine leukemia cells.

Table 2 shows the cytotoxic activity of **5a–c**. The results show that the substituent in the phenyl ring of hydroxycinnamic moieties affect the activity. In our previous reports, caffeamide compounds showed higher cytotoxic activity than coumaramide analogues [9, 10]. This is due to the presence of more phenolic groups in caffeamide than in *p*-coumaramide and ferulamide. However, in this study we found that **5a** and **5c** showed higher cytotoxic activity than **5b**. One possible explanation of this fact is that **5a** and **5c** which has lower deprotonation state can penetrate into the cell deeper than **5b** [13]. The effect of amine moiety of hydroxycinnamide compounds against P388 murine leukemia cells can be predicted by comparing the IC_{50} values of both **5a** and **5b** to *N*-(*o*-tolyl) analogues. The IC_{50} of **5a** is lower than the IC_{50} value of the former compound (16.97 µg/mL), but the difference is not significant. However, *N*-(*o*-tolyl)caffeamide showed strong cytotoxic activity with IC_{50} of 0.91 µg/mL, which is contrast to **5b**. The facts indicate that both the hydroxycinnamic and amine moieties play crucial role in the cytotoxicity of hydroxycinnamide compounds.

In addition, when comparing compound **5a** and **5c**, there are a huge difference in their cytotoxic activity. Both compounds have only a difference in the substituent on C-8, which **5c** has a methoxy group while no substituent in **5a**. This substituent caused the presence of intramolecular hydrogen bond between H atom of hydroxyl group and O atom of methoxy group in **5c**. Due to this hydrogen bond, the ability of **5c** to release a radical hydrogen is decrease, thus the cytotoxic activity of this compound is lower than **5a**.

Docking study

Docking analysis of compounds **5a**, **5b**, and **5c** was done through Autodock4 program. Result of analysis as shown in Table 3 with compound **5b** have a smaller binding energy than the others compound. This phenomenon is due to the more hydrogen bond interactions of **5b** against Top1 receptor. Visualization of interaction between **5b** and Top1 is shown in Fig. 2. The presence of two hydroxyl groups in compound **5b** causes this compound to have more hydrogen bonds then resulted a greater binding energy value than **5a** and **5c**. In 3D visualization, it was shown that the position of two hydroxyl groups as a donor hydrogen bond meet very close to the site of acceptor hydrogen bond at the Top1 surface.

Nevertheless, the interesting part is the inappropriate value of the inhibition activity with the resulting binding energy data. Compound **5b** has the lowest inhibition activity compared to **5a** and **5c** but has the highest bonding energy to Top1 proteins. To know the reason of this phenomenon,

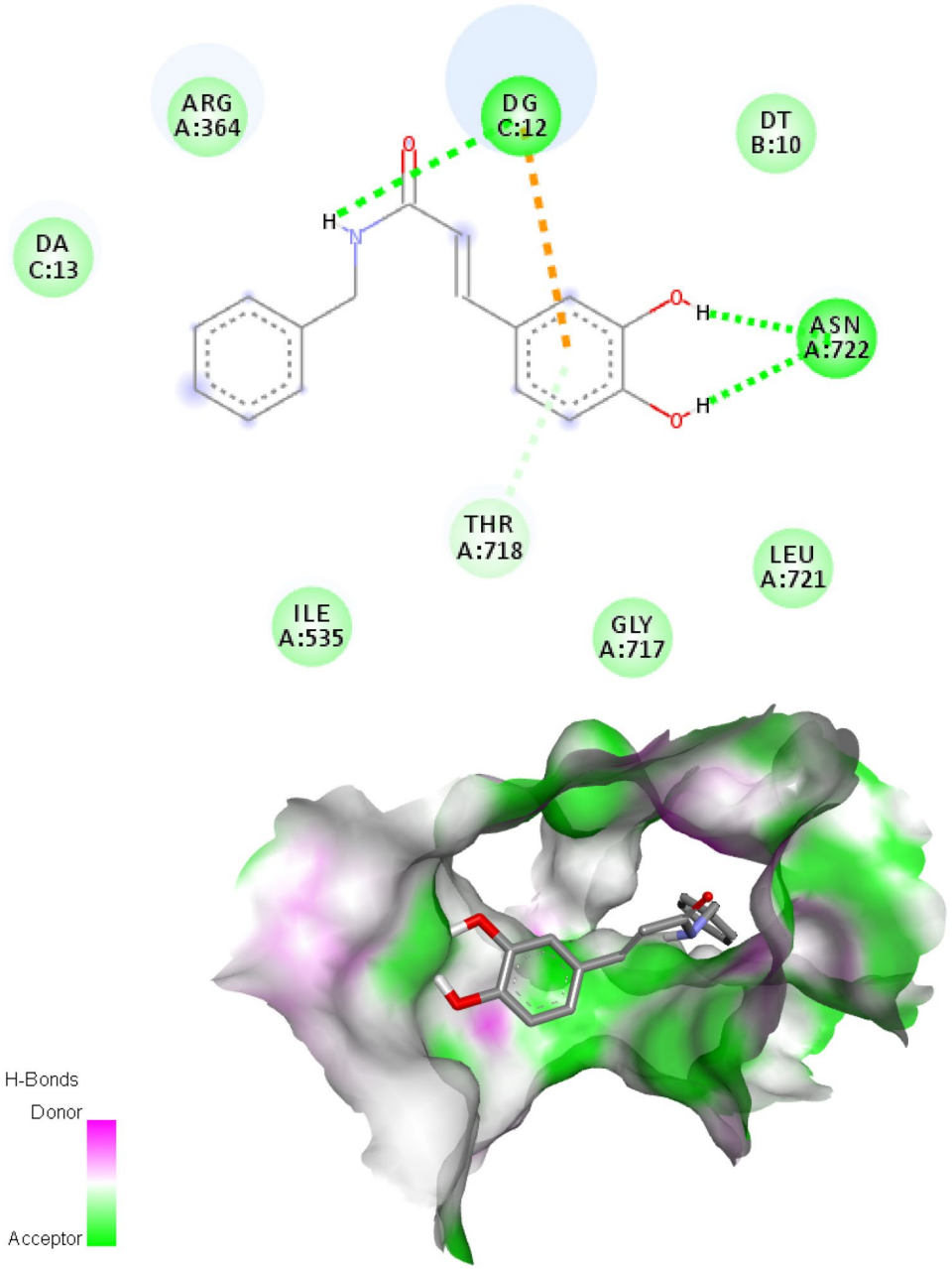


Fig. 2 2D and 3D Visualization of compound **5b** against Top1 protein receptor

Table 2 The cytotoxicity of synthesized compounds against leukemia murine P388 cell

Compound	IC_{50} value (μg/mL)
5a	16.15
5b	674.38
5c	179.56

Table 3 Docking analysis result of compounds **5a**, **5b**, and **5c** against Top1

Compound	ΔG (kcal/mol)	Ki (μM)	Hydrogen bond interaction
5a	−6.40	20.43	Asn722; DG12
5b	−6.64	13.47	Asn722; Asn722; DG12
5c	−6.62	14.08	Asn722; DG12

we compared compound **5b** and trans-*N*-(*o*-tolyl)caffeamide (IC_{50} = 0.91 μg/mL) by hydrophobic analysis of that has been synthesized [9] using LigPlot + program. Figure 3 showed the result of hydrophobic analysis of these two compounds. These two compounds have a two hydroxyl groups in phenyl ring but has a very difference inhibition activity value. Hydrophobic interactions of compound **5b** was resulted in Leu721, Gly717, Dt10, Da13, Thr718, and Arg364. Meanwhile, compound *trans-N*-(*o*-tolyl)caffeamide has at least three interaction at residue Dt10, Da113, and Dc112 for

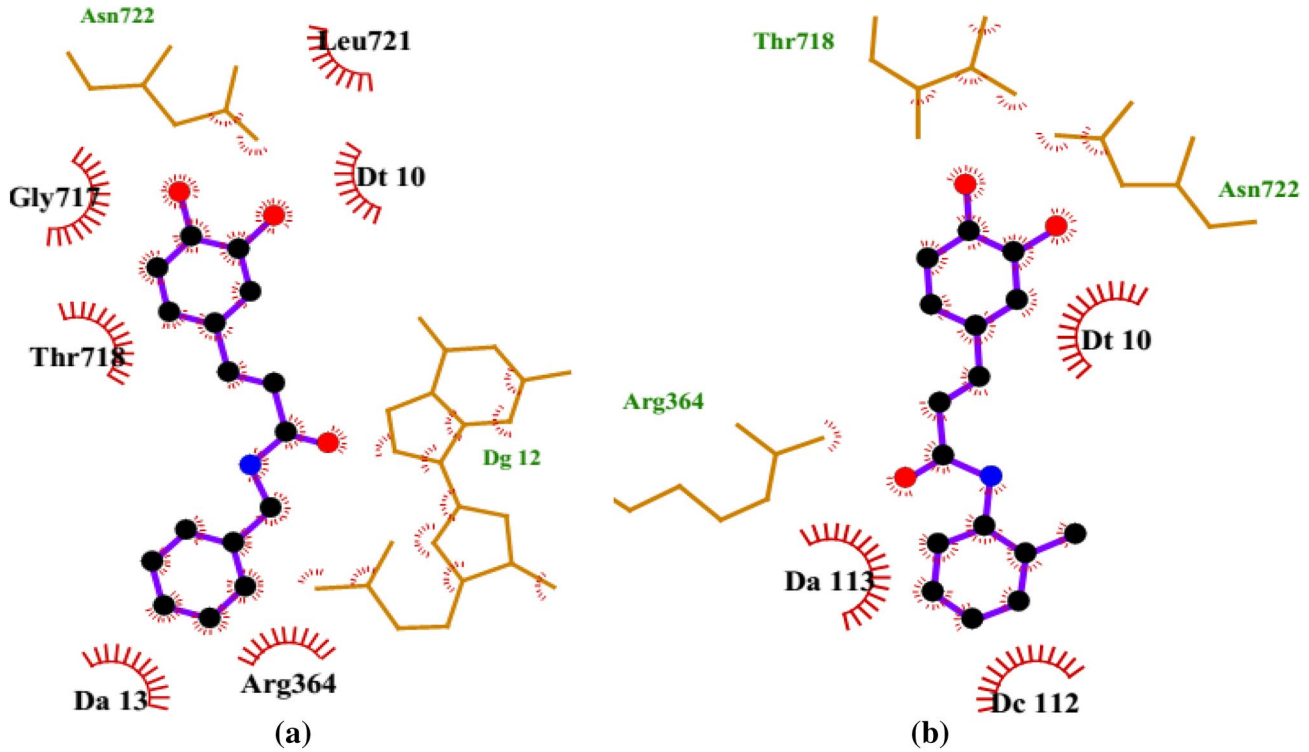


Fig. 3 Hydrophobic analysis result of compound **a 5b** and **b** trans-*N*-(*o*-tolyl)caffeamide against Top1

hydrophobic and hydrogen bond interactions also found at residue Arg364 and Thr718 while in **5b** these two residue only has hydrophobic interaction. It cause of *trans-N*-(*o*-tolyl)caffeamide having a better activity than **5b** because hydrogen bond interaction with Arg364 has an important role in inhibition mechanism of Top1 receptor [37].

Conclusion

Compound **5a–5c** have been successfully synthesized from hydroxycinnamic acids by indirect method. Compound **5a** showed moderate cytotoxicity against murine leukemia P388 cells, thus it has a highly potential to be used as an anticancer drug.

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Declarations

Conflict of interest All authors declare no conflict of interest.

References

1. L.H. Boudreau, J. Maillet, L.M. Leblanc, T.M. Jean-franc, Caffeic acid phenethyl ester and its amide analogue are potent inhibitors of leukotriene biosynthesis in human polymorphonuclear leukocytes. PLoS ONE **7**(2), 31833 (2012). https://doi.org/10.1371/journal.pone.0031833
2. M.G. Chochkova, E.Y. Chorbadzhiyska, G.I. Ivanova, H. Najdenski, M. Ninova, T.S. Milkova, Antimicrobial and radical scavenging activities of *N*-hydroxycinnamoyl-L-antimicrobial and radical scavenging activities of *N*-Hydroxycinnamoyl-L-cysteine and -L-proline ethyl esters. Nat. Prod. J. **2**(1), 1–5 (2012). https://doi.org/10.2174/2210315511202010050
3. M.G. Chochkova, P.P. Petrova, B.M. Stoykova, G.I. Ivanova, M. Sticha, G. Dibo, T.S. Milkova, Structure-activity relationships of n-cinnamoyl and hydroxycinnamoyl amides on α-glucosidase inhibition. J. Chem. **2017**, 1–5 (2017)
4. C. Cui, Z.P. Wang, X.J. Du, L.Z. Wang, S.J. Yu, X.H. Liu, Z.M. Li, W.G. Zhao, Synthesis and antiviral activity of hydrogenated ferulic acid derivatives. J. Chem. **2013**, 1–5 (2013)
5. D. Systemes, *Biovia discovery studio visualizer* (Dassault Systemes, San Diego, 2019)
6. M. De Armas-ricard, E. Ruiz-reyes, O. Ramírez-rodríguez, Caffeates and caffeamides: synthetic methodologies and their antioxidant properties. Int. J. Med. Chem. **2019**, 1–15 (2019)
7. H.D.R. Firdaus, T. Naid, N.H. Soekamto, S. Sumarna, M.F. Islam, Synthesis of piperidine and morpholine amides of ferulic acid and their bioactivity against P-388 leukemia cells. Int. J. ChemTech Res. **10**(1), 27–33 (2017)
8. S. Firdaus, D. Husain, H. Rasyid, Sukarti, Methylation of p-coumaric acid with dimethyl sulfate and sodium hydoxide as catalyst. Proc. First Int. Conf. Sci. **1**, 339–344 (2014)

9. Firdaus, Seniwati, N. Alamsyah, S. Paramita, Synthesis and activity of N-(o-tolyl)caffeamide and *N*-(o-tolyl)-p-coumaramide against P388 leukemia murine cells. J. Phys. Conf. Ser. **1341**(3), 032005 (2019)
10. S.N.H. Firdaus, S. Firdausiah, H. Rasyid, N. Asmi, M. Waelulu, Novel hydroxycinnamamide from morpholine and pyrrolidine: synthesis, characterization, docking study, and anticancer activity against P388 leukemia murine cells. J. Appl. Pharm. Sci. **11**(1), 40–48 (2021). https://doi.org/10.7324/JAPS.2021.110104
11. Firdaus, N.H. Soekamto, Seniwati, M.F. Islam, Sultan, Phenethyl ester and amide of ferulic acids: synthesis and bioactivity against p388 leukemia murine cells. J. Phys. Conf. Ser. **979**, 012016 (2018)
12. L. Georgiev, M. Chochkova, G. Ivanova, H. Najdenski, M. Ninova, T. Milkova, Radical scavenging and antimicrobial activities of cinnamoyl amides of biogenic monoamines. La Riv. Ital. Delle Sostanze Grasse **89**(2), 91 (2012)
13. B. Godlewska-żyłkiewicz, R. Swisloscka, M. Kalinowska, A. Golonko, G. Swiderslo, Z. Arciszewska, E. Nalewajko-Sielowoniuk, M. Naumowies, W. Lewandowski, Biologically active compounds of plants: structure-related antioxidant, microbiological and cytotoxic activity of selected carboxylic acid. Materials **13**(19), 1–37 (2020). https://doi.org/10.3390/ma13194454
14. J.D. Guzman, Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. Molecules **19**, 19292–19349 (2014). https://doi.org/10.3390/molecules191219292
15. M.D. Hanwell, D.E. Curtis, D.C. Lonie, T. Vandermeersch, E. Zurek, G.R. Hutchison, Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J. Cheminform. **4**, 1–17 (2012)
16. R.F. Helm, J. Ralph, R.D. Hatfield, Synthesis of feruloylated methyl glycosides and p-coumaroylated. Carbohydr. Res. **229**, 183–194 (1992)
17. R. Huey, G.M. Morris, A.J. Olson, D.S. Goodsell, A semiempirical free energy force field with charge-based desolvation. J. Comput. Chem. **28**(6), 1145–1152 (2007)
18. P.A. Jackson, J.C. Widen, D.A. Harki, K.M. Brummond, Covalent modifiers: a chemical perspective on the reactivity of α, β-unsaturated carbonyls with thiols via hetero-Michael addition reactions. J. Med. Chem. **60**(3), 839–885 (2017). https://doi.org/10.1021/acs.jmedchem.6b00788
19. S.M. Jakoveti, B.Z. Jugovi, M.M. Gvozdenovi, D.I. Bezbradica, Synthesis of aliphatic esters of cinnamic acid as potential lipophilic antioxidants catalyzed by lipase B from *Candida antarctica*. Appl Biochem Biotechnol **170**, 1560–1573 (2013). https://doi.org/10.1007/s12010-013-0294-z
20. A. Jitareanu, G. Tataringa, C. Tuchilus, M. Balan, U. Stanescu, Cinnamic acid derivatives and 4-aminoantipyrine amides—synthesis and evaluation of biological properties. Res. J. Chem. Sci. **3**(3), 9–13 (2013)
21. A. Khatkar, A. Nanda, P. Kumar, B. Narasimhan, Synthesis, antimicrobial evaluation and QSAR studies of p-coumaric acid derivatives. Arab. J. Chem. **10**, 3804–3815 (2017). https://doi.org/10.1016/j.arabjc.2014.05.018
22. B. Korošec, M. Sova, S. Turk, N. Krasevec, M. Novak, L. Lah, J. Stojan, B. Pobobnik, S. Berne, N. Zupanec, M. Bunc, S. Gobec, R. Komel, Antifungal activity of cinnamic acid derivatives involves inhibition of benzoate 4-hydroxylase (CYP53). J. Appl. Microbiol. **116**(4), 955–966 (2014). https://doi.org/10.1111/jam.12417
23 Y. Kuo, W. Jim, L. Su, C. Chung, C. Lin, Caffeic acid phenethyl ester is a potential therapeutic agent for oral cancer. Int. J. Mol. Sci. **16**, 10748–10766 (2015). https://doi.org/10.3390/ijms160510748
24. H. Kuncoro, L. Rijai, E. Julaeha, U. Supratman, Cytotoxic activity against P-388 murine Leukemia cell from Lygodium microphyllum herb. J. Farm. Galen. **3**(1), 13–16 (2003)
25. R.A. Laskowski, M.B. Swindells, LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. J. Chem. Inf. Model. **51**(10), 2778–2786 (2011)
26. G.E. Magoulas, D. Papaioannou, Bioinspired syntheses of dimeric hydroxycinnamic acids (Lignans) and hybrids, using phenol oxidative coupling as key reaction, and medicinal significance thereof. Molecules **19**, 19769–19835 (2014). https://doi.org/10.3390/molecules191219769
27. T. Matsubara, S. Asako, L. Ilies, E. Nakamura, Synthesis of anthranilic acid derivatives through iron-catalyzed ortho amination of aromatic carboxamides with N-chloroamines. J. Am. Chem. Soc. **136**, 646–649 (2014)
28. G. Morris, R. Huey, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J. Comput. Chem. **30**(16), 2785–2791 (2009). https://doi.org/10.1002/jcc.21256.AutoDock4
29. T. Nakazawa, K. Ohsawa, Metabolism of rosmarinic acid in rats. J. Nat. Prod. **2**(98), 993–996 (1998)
30. S.B. Nimse, D. Pal, A. Mazumder, R. Mazumder, Synthesis of cinnamanilide derivatives and their antioxidant and antimicrobial activity. J. Chem. **2015**, 1–6 (2015)
31. W. Peng, J.G. Wu, Y.B. Jiang, Y.J. Liu, T. Sun, N. Wu, C.J. Wu, Antitumor activity of 4-O-(2″-O-acetyl-6″-O-*p*-coumaroyl-*β*-d-glucopyranosyl)-*p*-coumaric acid against lung cancers via mitochondrial-mediated apoptosis. Chem. Interact. J. **233**, 8–13 (2015). https://doi.org/10.1016/j.cbi.2015.03.014
32. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. **25**, 1605–1612 (2004)
33. K. Saritha, G. Rajitha, Synthesis, antimicrobial and antioxidant activity of *N*-dimethylaminophenylallylidene cinnamide derivatives. Int. Res. J. Pharm. **9**(12), 120–127 (2018). https://doi.org/10.7897/2230-8407.0912304
34. A. Rauf, H. Parveen, Direct esterification of fatty acids with phenylalkanols by using dicyclohexylcarbodiimide. Eur. J. Lipid Sci. Technol. **106**, 97–100 (2004). https://doi.org/10.1002/ejlt.200300887
35 S. Salahuddin, M. Hanafi, H. Hariyanti, Synthesis and anticancer activity test of 2-hydroxy-n-phenylnicotinamide. Indones. J. Chem. **13**(2), 166–170 (2013)
36. U.Y. Shaheen, P-coumaric acid ester with potential antioxidant activity from the genus salvia. Free Radic. Antioxid. **1**(1), 23–27 (2011). https://doi.org/10.5530/ax.2011.1.5
37. B.L. Staker, C.M. FeeseMD, Y. Pommier, D. Zembower, L. Stewart, A.B. Burgin, Structures of three classes of anticancer agents bound to the human topoisomerase I-DNA covalent complex. J. Med. Chem. **48**(7), 2336–2345 (2005). https://doi.org/10.1021/jm049146p
38. K. Takao, K. Toda, T. Saito, Y. Sugita, Synthesis of amide and ester derivatives of cinnamic acid and its analogs: evaluation of their free radical scavenging and monoamine oxidase and cholinesterase inhibitory activities. Chem. Pharm. Bull. **65**(11), 1020–1027 (2017)
39 P. Tang, *Boric Acid Catalyzed Amide Formation from Carboxylic Acids and Amines: N-Benzyl-4-Phenylbutyramide. Organic Syntheses* (Wiley, Hoboken, 2005), pp. 262–272
40. O. Taofiq, A.M. González-Paramás, M.F. Barreiro, I.C.F.R. Ferreira, Hydroxycinnamic acids and their derivatives: future perspectives, a review. Molecules **22**(281), 1–24 (2017). https://doi.org/10.3390/molecules22020281
41. J. Teixeira, A. Gaspar, E.M. Garrido, J. Garrido, F. Borges, Hydroxycinnamic acid antioxidants: an electrochemical overview. Biomed. Res. Int. **2013**, 1–11 (2013)

42. T. Tokoroyama, Discovery of the Michael reaction. Eur. J. Org. Chem. **2010**, 2009–2016 (2010). https://doi.org/10.1002/ejoc.200901130
43. G. Wang, Q.Y. Yu, J. Wang, S. Wang, S.Y. Chen, X.Q. Yu, Iodide-catalyzed amide synthesis from alcohols and amines. RSC Adv. **3**(44), 21306–21310 (2013). https://doi.org/10.1039/C3RA43799J
44. Y. Xiao, X. Yang, B. Li, H. Yuan, S. Wan, Y. Xu, Design, synthesis and antifungal/insecticidal evaluation of novel cinnamide derivatives. Molecules **16**, 8945–8957 (2011). https://doi.org/10.3390/molecules16118945
45. Y.H. Yang, T. Raku, E. Song, S.H. Park, D. Yoo, H.Y. Park, B.G. Kim, H.J. Kim, S.H. Lee, H.S. Kim, Y. Tokiwa, Lipase catalyzed reaction of L-ascorbic acid with cinnamic acid esters and substituted cinnamic acids. Biotechnol. Bioprocess Eng. **17**, 50–54 (2012). https://doi.org/10.1007/s12257-011-0071-1
46. K. Zhou, D. Chen, B. Li, B. Zhang, F. Miao, L. Zhou, Bioactivity and structure-activity relationship of cinnamic acid esters and their derivatives as potential antifungal agents for plant protection. PLoS ONE **12**(4), 0176189 (2017)